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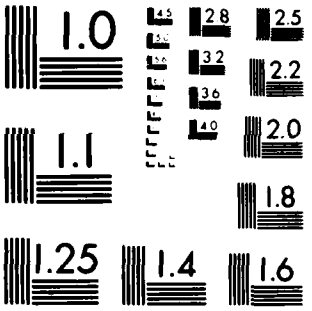

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EVALUATION STUDIES OF THE DEN-2/S-1 VACCINE

Annual and Final Report

Edmundo Kraiselburd, Ph.D.

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DEN-2 and -4 viruses dose rhesus monkeys seroconversion

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The purpose of this study was to determine how much dengue 2 and dengue 4 viruses were required to infect rhesus monkeys. To this effect, groups of five dengue antibody free animals were inoculated subcutaneously with serial 10 fold dilutions of dengue 2 (PR159) and dengue 4 (H241) viruses. Virus titrationswere carried out simultaneously by direct plaque assay in LLC-MK_2 cells, by mosquito inoculation using both Aedes aegypti and Toxorynchites amboiensis and in the monkeys. Blood samples for serology were taken at 30

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20. Abstract (continuation)

and 60 days post infection. Neutralization tests revealed that 9.5 mosquito infectious dose$_{50}$ (MID)$_{50}$ of dengue 2 and 22 MID$_{50}$ of dengue 4 were required to infect 50% of the monkeys. The data suggest that in recipients challenges with wild type virus, a minimum of 100 MID$_{50}$'s should be used as the virus challenge dose. (Kraiselburd, E. and Gubler, D., Manuscript in Preparation

Accomplishments: A group of 31 tuberculine-negative juvenile rhesus monkeys were pretested for neutralizing antibodies to dengue-2 and 4 serotypes. All animals were found to be free of dengue virus antibody. All monkeys were housed at the Caribbean Primate Research Center (Sabana Seca) in individual steel squeeze cages in a mosquito-proof area, 15 days before the pretest bleeding was performed. Groups of 5 animals were inoculated with serial dilutions of either dengue-2 (PR159) or dengue-4 (H241) virus preparations.

Dengue-2 virus (geometric mean titer 2.8×10^4 pfu/ml; 3.0×10^6 MID_{50}/ml) was serially diluted to 1/5 10^{-5}, 1/5 10^{-6}, 1/5 10^{-7}. Group 1 monkeys were s.c. inoculated with 0.5 ml of 1/5 $\times 10^{-5}$ dilution (5.6×10^{-2} pfu/ml; 6.0 MID_{50}/ml). Group 2 and group 3 monkeys were s.c. inoculated with 0.5 ml of 1/5 10^{-6} dilution 5.6×10^{-3} pfu/ml; 6.0×10^{-1} MID_{50}/ml) and 1/5 $\times 10^{-7}$ dilution (5.6×10^{-4} pfu/ml; 6.0×10^{-2} MID_{50}/ml), respectively (see Table II). Dengue-4 virus (geometric mean titer 7.2×10^2 pfu/ml; 1.5×10^6 MID_{50}/ml) was diluted to 1/5 10^{-4}, 1/5 10^{-5} and 1/5 10^{-6}. Groups 4, 5 and 6 monkeys were s.c. inoculated with 0.5 ml of 1/5 10^{-5}, 1/5 10^{-6} and 1/5 10^{-7} dilutions of dengue-4 respectively (see table I). One monkey was used as uninoculated control. Animal inoculation was performed on April 13, 1982.

MID_{50} (mosquito infection dose 50) were determined using both Aedes Aegypti and Toxorynchitis mosquitoes by Dr. Gubler (San Juan Laboratories, CDC, Puerto Rico) before and after animal inoculation. MID_{50} titers reported are geometric means of three independent MID_{50} determinations.

Animals were bled on post infection days 30 and 60. Seroconversion to the respective dengue virus type was determined by N test performed at 35°C for 30 minutes. Two monkeys (B766 and B773) died on post infection day 30. An autopsia revealed that the animals died of suffocation secondary to Kentamine sedation. Histopathological review of the tissues confirmed that the animals died of complications of recovery from sedation.

Only one group 1 monkey seroconverted. The remaining dengue-2 infected monkeys did not mount an antibody response to the diluted virus inoculum. Only 2 group 4 monkeys and only 1 group 5 monkeys mounted a positive antibody response to dengue-4 virus.

The minimum dose of dengue virus required for animal seroconversion could not be determined by this experiment (see Quarterly Report, June 30, 1982). To determine this virus dose, lower virus dilutions were used in the experiments described below.

On infection day (June 15, 1982), dengue-2 and dengue-4 viruses were titered again. The titers obtained with the newly defrosted virus samples were 2.5×10^4 pfu/ml (dengue-2) and 800 pfu/ml (dengue-4).

Since on April 13, the virus titers obtained were 3.2×10^4 pfu/ml (dengue-2) and 6.5×10^2 pfu/ml (dengue-4), geometric mean titers (GMT) were calculated for both virus preparations. GMT of 2.8×10^4 pfu/ml and 720 pfu/ml were obtained from dengue-2 and dengue-4, respectively. GMT's were also obtained from three independent MID_{50} determinations (performed by Dr. Gubler, CDC, San Juan). The MID_{50} titers obtained were 3.0×10^6 MID_{50}/ml (dengue-2) and 1.5×10^6 MID_{50}/ml (dengue-4). These GMT were used for calculating monkey ID_{50} from data presented on tables I and II.

On June 15, 1982 groups 2 and 3 monkeys (previously inoculated with diluted dengue-2) were s.c. infected with 0.5 ml of 1.44 pfu/ml and 0.144 pfu/ml of dengue-4, respectively. Groups 6 and 5 monkeys (previously inoculated with diluted dengue-4) were s.c. infected with 0.5 mls of 5.6 pfu/ml and 0.56 pfu/ml of dengue-2 respectively. Infected monkeys were bled on post infection days 30 and 60 and their N antibody titers determined. Results from these experiments are presented in tables I and II.

TABLE I

Immune Response of Rhesus Monkeys to a serially diluted dengue-4 (H24) Virus Preparation.

Dengue 4 Virus Dose pfu	MID_{50}	Seroconversion	Geometric N Titers 30d	60d
7.2×10^{-1}	3.5×10^{3}	5/5	46	> 160
7.2×10^{-2}	3.5×10^{2}	4/5	60	>160
7.2×10^{-3}	3.5×10^{1}	2/5	49	160
7.2×10^{-4}	3.5	1/5	< 10	160
7.2×10^{-5}	3.5×10^{-1}	0/5	< 10	< 10

Conclusion: For dengue 4, 22 mosquito $1D_{50}$ (10^{-2} pfu) are equivalent to 1 monkey ID_{50}

TABLE II

Immune Response of Rhesus Monkeys to a Serially Diluted Dengue-2 (PR159) Virus Preparation

Dengue 2 Virus Dose pfu	MID50	Seroconversion	Geometric N Titers 30d	60d
2.8	3×10^{2}	5/5	>160	>160
2.8×10^{-1}	3×10^{1}	4/5	>160	>160
2.8×10^{-2}	3	1/5	>10	>640
2.8×10^{-3}	3×10^{-1}	0/5	<10	<10
2.8×10^{-4}	3×10^{-2}	0/5	<10	<10

Conclusion: For dengue 2, 9.5 mosquito $1D_{50}$ (13×10^{-2} pfu) are equivalent to 1 monkey ID_{50}.

Problems: Viruses received from WRAIR were supposed to have 100x higher titers than the one obtained in our laboratory. Therefore, the viruses may have lost titer after shipment from WRAIR to Puerto Rico. This may be of importance in future experiments.

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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